

INTERNATIONAL COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

01 December 1999 (01.12.99)

International application No.

PCT/AU99/00306

Applicant's or agent's file reference

91641-1 check

International filing date (day/month/year)

23 April 1999 (23.04.99)

Priority date (day/month/year)

23 April 1998 (23.04.98)

Applicant

CLARK, Susan, Joy et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

11 November 1999 (11.11.99)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 95/09050

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C12N9/10 A61K48/00 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROC. NATL. ACAD. SCI., vol. 85, no. 14, September 1988 NATL. ACAD SCI., WASHINGTON, DC, US;, pages 6518-6522, J.A. MOSCOW ET AL. 'Isolation of the human anionic glutathione S-transferase cDNA and the reaction of its gene expression to estrogen-receptor content in primary breast cancer' see the whole document --- -/--	24



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

7 November 1995

Date of mailing of the international search report

30. 11. 95

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

PCT/US 95/09050

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROC. NATL. ACAD. SCI., vol. 89, no. 6, 15 March 1992 NATL. ACAD SCI., WASHINGTON, DC, US;, pages 2394-2398, H.J. PROCHASKA ET AL. 'Rapid detection of inducers of enzymes that protect against carcinogens' cited in the application see the whole document ---	19
X	PROC. NATL. ACAD. SCI., vol. 90, no. 7, 1 April 1993 NATL. ACAD SCI., WASHINGTON, DC, US;, pages 2965-2969, T. PRESTERA ET AL. 'Chemical and molecular regulation of enzymes that detoxify carcinogens' cited in the application see the whole document ---	19
A	CHEMICAL ABSTRACTS, vol. 116, no. 19, 11 May 1992 Columbus, Ohio, US; abstract no. 190180, KAWADA, YUKIMICHI ET AL. 'Method and reagents for blood glutathione S - transferase immunoassay and its use in clinical diagnosis' see abstract & JP, A, 04 025 763 (MARUKO PHARMACEUTICAL CO., LTD., JAPAN; DAINIPPON PHARMACEUTICAL CO., L) ---	1, 3, 8, 11-15
A	WO, A, 90 12088 (THE UNITED STATES OF AMERICA) 18 October 1990 see the whole document ---	1, 3, 8, 11-15
A	J UROL 150 (1). 1993. 209-214. CODEN: JOURAA ISSN: 0022-5347, July 1993 RIPPLE M ET AL. 'CHARACTERISTICS OF THE GLUTATHIONE GLUTATHIONE - S - TRANSFERASE DETOXIFICATION SYSTEM IN MELPHALAN RESISTANT HUMAN PROSTATE CANCER CELLS.' see the whole document ---	1-30

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INTERNATIONAL SEARCH REPORT

 Int. Application No.
 PCT/US 95/09050

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>85TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN FRANCISCO, CALIFORNIA, USA, APRIL 10-13, 1994. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 35 (0). 1994. 293. ISSN: 0197-016X, ABSTRACT NO. 1746, BUETOW K H ET AL 'GSTM1 and risk for breast and prostate cancer.' see abstract</p> <p>---</p>	1-30
A	<p>EIGHTY-EIGHTH ANNUAL MEETING OF THE AUA (AMERICAN UROLOGICAL ASSOCIATION), SAN ANTONIO, TEXAS, USA, MAY 15-20, 1993. J UROL 149 (4 SUPPL.). 1993. 470A. CODEN: JOURAA ISSN: 0022-5347, ABSTRACT NO.1028, BROOKS J D ET AL 'ALTERED REGULATION OF GLUTATHIONE - S - TRANSFERASE PI IN HUMAN PROSTATIC CANCER CELLS.' see abstract</p> <p>---</p>	1-30
A	<p>85TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN FRANCISCO, CALIFORNIA, USA, APRIL 10-13, 1994. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 35 (0). 1994. 379. ISSN: 0197-016X, ABSTRACT NO. 2258, CANADA A T ET AL 'Glutathione and glutathione S - transferase content of prostate cancer cell lines and prostate tissue.' see abstract</p> <p>---</p>	1-30
A	<p>CRC CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 27, no. 4,5, 1992 CRC PRESS, INC., USA, pages 337-384, S.TSUCHIDA AND K. SATO 'Glutathione transferases and cancer' cited in the application see the whole document</p> <p>---</p>	1-30
P,X	<p>PROC. NATL. ACAD. SCI. U. S. A. (1994), 91(24), 11733-7 CODEN: PNASA6;ISSN: 0027-8424, 22 November 1994 LEE, WEN-HSIANG ET AL 'Cytidine methylation of regulatory sequences near the pi.-class glutathione S - transferase gene accompanies human prostatic carcinogenesis' see the whole document</p> <p>---</p>	1-30

INTERNATIONAL SEARCH REPORT

Int. Patent Application No.
PCT/US 95/09050

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>NINETIETH ANNUAL MEETING OF THE AMERICAN UROLOGICAL ASSOCIATION, LAS VEGAS, NEVADA, USA, APRIL 23-28, 1995. JOURNAL OF UROLOGY 153 (4 SUPPL.). 1995. 446A. ISSN: 0022-5347, ABSTRACT NO. 869, LEE W-H ET AL 'Cytidine methylation of the pi-class glutathione S - transferase gene promoter in human prostate cancer.' see abstract</p> <p style="text-align: center;">---</p>	1-30
P,X	<p>EIGHTY-SIXTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, TORONTO, ONTARIO, CANADA, MARCH 18-22, 1995. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 36 (0). 1995. 538. ISSN: 0197-016X, ABSTRACT NO. 3206, LEE W-H ET AL 'Methylation of 5' regulatory sequences near the GSTP1 gene in human prostatic carcinomas.' see abstract</p> <p style="text-align: center;">-----</p>	1-30

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 95/09050

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 19-23, 27 and 24-26, 28-30 (partially), (as far as in vivo methods are concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9012088	18-10-90	AU-B- 5435990	05-11-90

said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) determining the presence of amplified DNA.

Since the amplification is designed to only amplify the target region if
5 the said site or sites at which abnormal cytosine methylation (i.e. as compared to the corresponding site or sites of DNA from subjects without the disease or condition being assayed) occurs is/are methylated, the presence of amplified DNA will be indicative of the disease or condition in the subject from which the isolated DNA has been obtained. The assay thereby provides
10 a means for diagnosing or prognosing the disease or condition in a subject.

The step of isolating DNA may be conducted in accordance with standard protocols. The DNA may be isolated from any suitable body sample, such as cells from tissue (fresh or fixed samples), blood (including serum and plasma), semen, urine, lymph or bone marrow. For some types of
15 body samples, particularly fluid samples such as blood, semen, urine and lymph, it may be preferred to firstly subject the sample to a process to enrich the concentration of a certain cell type (e.g. prostate cells). One suitable process for enrichment involves the separation of required cells through the use of cell-specific antibodies coupled to magnetic beads and a magnetic cell
20 separation device.

Prior to the amplifying step, the isolated DNA is preferably treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming
25 a base pair with guanine. This treatment permits the design of primers which enable the selective amplification of the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated.

Preferably, following treatment and amplification of the isolated DNA, a test is performed to verify that unmethylated cytosines have been
30 efficiently converted to uracil or another nucleotide capable of forming a

base pair with adenine, and that methylated cytosines have remained unchanged or efficiently converted to another nucleotide capable of forming a base pair with guanine.

Preferably, the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite in accordance with standard protocols. As will be clear from the above discussion of bisulphite treatment, unmethylated cytosines will be converted to uracil whereas methylated cytosines will be unchanged. Verification that unmethylated cytosines have been converted to uracil and that methylated cytosines have remained unchanged may be achieved by;

- (i) restricting an aliquot of the treated and amplified DNA with a suitable restriction enzyme(s) which recognise a restriction site(s) generated by or resistant to the bisulphite treatment, and
 - (ii) assessing the restriction fragment pattern by electrophoresis.
- Alternatively, verification may be achieved by differential hybridisation using specific oligonucleotides targeted to regions of the treated DNA where unmethylated cytosines would have been converted to uracil and methylated cytosines would have remained unchanged.

The amplifying step may involve polymerase chain reaction (PCR) amplification, ligase chain reaction amplification (20) and others (21).

Preferably, the amplifying step is conducted in accordance with standard protocols for PCR amplification, in which case, the reactants will typically be suitable primers, dNTPs and a thermostable DNA polymerase, and the conditions will be cycles of varying temperatures and durations to effect alternating denaturation of strand duplexes, annealing of primers (e.g. under high stringency conditions) and subsequent DNA synthesis.

To achieve selective PCR amplification with bisulphite-treated DNA, primers and conditions may be used to discriminate between a target region including a site or sites of abnormal cytosine methylation and a target region where there is no site or sites of abnormal cytosine methylation. Thus, for

amplification only of a target region where the said site or sites at which abnormal cytosine methylation occurs is/are methylated, the primers used to anneal to the bisulphite-treated DNA (i.e. reverse primers) will include a guanine nucleotide(s) at a site(s) at which it will form a base pair with a methylated cytosine(s). Such primers will form a mismatch if the target region in the isolated DNA has unmethylated cytosine nucleotide(s) (which would have been converted to uracil by the bisulphite treatment) at the site or sites at which abnormal cytosine methylation occurs. The primers used for annealing to the opposite strand (i.e. the forward primers) will include a cytosine nucleotide(s) at any site(s) corresponding to site(s) of methylated cytosine in the bisulphite-treated DNA.

Preferably, the primers used for the PCR amplification are of 12 to 30 nucleotides in length and are designed to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed. In addition, the primers preferably include a terminal nucleotide that will form a base pair with a cytosine nucleotide (reverse primer), or the guanine nucleotide opposite (forward primer), that is abnormally methylated in the DNA of a subject with the disease or condition being assayed.

The step of amplifying is used to amplify a target region within the GST-Pi gene and/or its regulatory flanking sequences. The regulatory flanking sequences may be regarded as the flanking sequences 5' and 3' of the GST-Pi gene which include the elements that regulate, either alone or in combination with another like element, expression of the GST-Pi gene. Preferably, the regulatory flanking sequences consist of the 400 nucleotide sequence immediately 5' of the transcription start site and the 100 nucleotide sequence immediately 3' of the transcription stop site.

More preferably, the step of amplifying is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking

sequences defined by (and inclusive of) CpG sites -43 to +55 (wherein the numbering of the CpG sites is relative to the transcription start site). The numbering and position of CpG sites is shown in Figure 1.

5 The step of determining the presence amplified DNA may be conducted in accordance with standard protocols. One convenient method involves visualisation of a band(s) corresponding to amplified DNA, following gel electrophoresis.

10 Preferably, the disease or condition to be assayed is selected from cancers, especially hormone dependent cancers such as prostate cancer, breast cancer and cervical cancer, and liver cancer.

For the diagnosis or prognosis of prostate cancer, the step of amplifying preferably amplifies a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53, more preferably, -43 to +10. However, within these target regions it is believed that there are CpG sites which show variability in methylation status in prostate cancer or are methylated in other tissues. Thus, for the target region defined by (and inclusive of) CpG sites -43 to +10, it is preferred that the primers used for amplification be designed so as to minimise (i.e. by use of redundant primers or by avoidance of the sites) the influence of CpG sites -36, -32, -23, -20, -14 and a polymorphic region covering site -33. Further, for DNA isolated from cells other than from prostate tissue (e.g. blood), it is preferred that the primers used be designated to amplify a target region that does not include the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -7 to +7, or, more preferably, -13 to +8, since this may lead to false positives. Further preferred target regions, therefore, are within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14, -43 to -8, +9 to +53 and +1 to +53.

Suitable primer pairs for the diagnosis or prognosis of prostate cancer, include those consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers (i.e. anneal to the 5' end of the target region)

- | | | |
|----|-------------------------------|----------------|
| 5 | CGCGAGGTTTTCGTTGGAGTTTCGTCGTC | (SEQ ID NO: 1) |
| | CGTTATTAGTGAGTACGCGCGGTTC | (SEQ ID NO: 2) |
| | YGGTTTTAGGGAATTTTTTTTCGC | (SEQ ID NO: 3) |
| | YGGYGYGTTAGTTYGTTGYGTATATTC | (SEQ ID NO: 4) |
| | GGGAATTTTTTTTCGCGATGTTTYGGCGC | (SEQ ID NO: 5) |
| 10 | TTTTTAGGGGGTTYGGAGCGTTTC | (SEQ ID NO: 6) |
| | GGTAGGTIGYGTTTATCGC | (SEQ ID NO: 7) |

Reverse Primers (i.e. anneal to the extension of the forward primer)

- | | | |
|----|-----------------------------------|-----------------|
| | TCCCATCCCTCCCCGAAACGCTCCG | (SEQ ID NO: 8) |
| | GAAACGCTCCGAACCCCCTAAAAACCGCTAACG | (SEQ ID NO: 9) |
| 15 | CRCCCTAAAATCCCCRAAATCRCCGCG | (SEQ ID NO: 10) |
| | ACCCCRACRACCRCTACACCCCRACGTCG | (SEQ ID NO: 11) |
| | CTCTTCTAAAAAATCCCRCAACTCCCGCCG | (SEQ ID NO: 12) |
| | AAAACRCCCTAAAATCCCCGAAATCGCCG | (SEQ ID NO: 13) |
| | AACTCCCRCCGACCCCAACCCCGACGACCG | (SEQ ID NO: 14) |
| 20 | AAAAATTCRAATCTCTCCGAATAAACG | (SEQ ID NO: 15) |
| | AAAAACCRAAATAAAAACCACACGACG | (SEQ ID NO: 16) |

wherein Y is C, T or, preferably, a mixture thereof, and R is A, G or, preferably, a mixture thereof.

For the diagnosis or prognosis of liver cancer, the step of amplifying preferably amplifies a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14 and/or +9 to +53. However, within these target regions it is believed that there are CpG sites which show variability in methylation status in liver cancer or are methylated in other tissues. Thus, for the target region defined by (and inclusive of) CpG sites -43 to -14, it is preferred that the primers used

for amplification be designed so as to minimise (i.e. by use of redundant primers or by avoidance of the sites) the influence of CpG sites -36, -32, -23, -20, -14 and a polymorphic region covering site -33.

5 It will be appreciated by persons skilled in the art, that a site or sites of abnormal cytosine methylation within the above identified target regions of the GST-Pi gene and/or its regulatory flanking sequences, could be detected for the purposes of diagnosing or prognosing a disease or condition (particularly, prostate cancer and/or liver cancer) by methods which do not involve selective amplification. For instance, oligonucleotide/polynucleotide probes could be designed for use in hybridisation studies (e.g. Southern blotting) with bisulphite-treated DNA which, under appropriate conditions of stringency, selectively hybridise only to DNA which includes a site or sites of abnormal methylation of cytosine(s). Alternatively, an appropriately selected informative restriction enzyme(s) could be used to produce restriction
10 fragment patterns that distinguish between DNA which does and does not include a site or sites of abnormal methylation of cytosine(s).
15

Thus, in a second aspect, the present invention provides a diagnostic or prognostic assay for a disease or condition in a subject said disease or condition characterised by abnormal methylation of cytosine at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;
20

- (i) isolating DNA from said subject, and
- (ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.
25

The step of isolating DNA may be conducted as described above in relation to the assay of the first aspect.

Preferably, the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is selected from the regions defined by (and inclusive of)
30

CpG sites -43 to +53, -43 to +10, -43 to -14, +9 to +53 and +1 to +53. However, within these regions, it is preferred that certain sites (namely, CpG sites, -36, -33, -32, -23, -20, -17 and -14) be avoided as the site or sites at which, for the purpose of the assay, the presence of abnormal methylation of cytosine is determined.

Where the determination step is to involve selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probes, prior to the determination step, the isolated DNA is preferably treated (e.g. with bisulphite) such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine. This treatment permits the design of probes which allow for selective hybridisation to a target region including a site or sites of abnormal methylation of cytosine.

In a third aspect, the present invention provides a primer or probe (sequence shown in the 5' to 3' direction) comprising a nucleotide sequence selected from the group consisting of:

- | | | |
|----|----------------------------------|-----------------|
| | CGCGAGGTTTTCGTTGGAGTTTCGTCGTC | (SEQ ID NO: 1) |
| | CGTTATTAGTGAGTACGCGCGGTTC | (SEQ ID NO: 2) |
| 20 | YGGTTTTAGGGAATTTTTTTTCGC | (SEQ ID NO: 3) |
| | YGGYGYGTTAGTTYGTTGYGTATATTC | (SEQ ID NO: 4) |
| | GGAATTTTTTTTCGCGATGTTTYGGCGC | (SEQ ID NO: 5) |
| | TTTTTAGGGGGTTYGGAGCGTTTC | (SEQ ID NO: 6) |
| | GGTAGGTTGYGTTTATCGC | (SEQ ID NO: 7) |
| 25 | AAAAATTCRAATCTCTCCGAATAAACG | (SEQ ID NO: 8) |
| | AAAAACCRAAATAAAAACCACACGACG | (SEQ ID NO: 9) |
| | TCCCATCCCTCCCCGAAACGCTCCG | (SEQ ID NO: 10) |
| | GAAACGCTCCGAACCCCTAAAAACCGCTAACG | (SEQ ID NO: 11) |
| | CRCCCTAAAATCCCCRAAATCRCCGCG | (SEQ ID NO: 12) |
| 30 | ACCCCRACRACCRCTACACCCRAACGTCTG | (SEQ ID NO: 13) |

Claims:

1. A diagnostic or prognostic assay for a disease or condition in a subject,
5 said disease or condition characterised by abnormal methylation of cytosine
at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its
regulatory flanking sequences, wherein said assay comprises the steps of;
- (i) isolating DNA from said subject,
- (ii) exposing said isolated DNA to reactants and conditions for the
10 amplification of a target region of the GST-Pi gene and/or its regulatory
flanking sequences which includes a site or sites at which abnormal cytosine
methylation characteristic of the disease or condition occurs, the
amplification being selective in that it only amplifies the target region if the
said site or sites at which abnormal cytosine methylation occurs is/are
15 methylated, and
- (iii) determining the presence of amplified DNA.
2. An assay according to claim 1, wherein the amplifying step is used to
amplify a target region within the GST-Pi gene and/or its regulatory flanking
20 sequences, wherein the regulatory flanking sequences consist of the 400
nucleotide sequence immediately 5' of the transcription start site of the GST-
Pi gene and the 100 nucleotide sequence immediately 3' of the transcription
stop site of the GST-Pi gene.
- 25 3. An assay according to claim 1 or 2, wherein the amplifying step is used
to amplify a target region within the region of the GST-Pi gene and its
regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to
+55.
- 30 4. An assay according to any one of the preceding claims, wherein prior
to the amplifying step, the isolated DNA is treated such that unmethylated

cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.

5 5. As assay according to any one of the preceding claims, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

6. An assay according to claim 5, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the
10 reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

15 7. An assay according to claim 6, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.

20 8. An assay according to claim 7, wherein the primers are of 12 to 30 nucleotides in length.

9. An assay according to claim 8, wherein the primers are selected so as
25 to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.

10. An assay according to claim 4, wherein the treatment of the isolated
30 DNA involves reacting the isolated DNA with bisulphite.

11. As assay according to claim 10, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

5 12. An assay according to claim 11, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

10 13. An assay according to claim 12, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.

15 14. An assay according to claim 13, wherein the primers are of 12 to 30 nucleotides in length.

20 15. An assay according to claim 14, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.

25 16. An assay according to any one of the preceding claims, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.

30 17. An assay according to any one of the preceding claims, wherein the disease or condition to be assayed is selected from cancers.

18. An assay according to claim 17, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

5 19. An assay according to claim 18, wherein the disease or condition to be assayed is prostate cancer.

20. An assay according to claim 19, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its
10 regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53.

21. An assay according to claim 19, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its
15 regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +10.

22. An assay according to claim 19, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its
20 regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

23. An assay according to claim 19, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its
25 regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -8.

24. An assay according to claim 19, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its

regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

25. An assay according to claim 19, wherein the amplifying step is used to
5 amplify a target region within the region of the GST-Pi gene and its
regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to
+53.

26. An assay according to claim 19, wherein the amplifying step involves
10 PCR amplification using primer pairs consisting of a forward and reverse
primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCTGTC (SEQ ID NO: 1)
CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)
15 YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)
YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)
GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)
TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)
GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

20 Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)
GAAACGCTCCGAACCCCTAAAAACCGCTAACG (SEQ ID NO: 9)
CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)
ACCCCRACRACCRCTACACCCRAACGTCG (SEQ ID NO: 11)
25 CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 12)
AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)
AACTCCCRCCGACCCCAACCCGACGACCG (SEQ ID NO: 14)
AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)
AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

30 wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

27. An assay according to claim 19, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

5 CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCATCCCTCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

10

28. An assay according to claim 19, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

15 YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

20 ACCCCRACRACCRCTACACCCRAACGTCTG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCGAAATCGCCG (SEQ ID NO: 13)

AACCTCCCRCCGACCCCAACCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

25

29. An assay according to claim 19, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse

primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

5 Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

10 30. An assay according to claim 18, wherein the disease or condition to be assayed is liver cancer.

31. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its
15 regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

32. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its
20 regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

33. A diagnostic or prognostic assay for a disease or condition in a subject said disease or condition characterised by abnormal methylation of cytosine
25 at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;
(i) isolating DNA from said subject, and
(ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking
30 sequences defined by (and inclusive of) CpG sites -43 to +55.

34. An assay according to claim 33, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is selected from the regions defined by (and inclusive of) CpG sites -43 to +53, -43 to +10, -43 to -14, +9 to +53 and +1 to +53.

35. An assay according to claim 34, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +9 to +53.

36. An assay according to claim 34, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +1 to +53.

37. An assay according to any one of claims 33 to 36, wherein prior to the determination step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.

38. An assay according to claim 37, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

39. An assay according to any one of claims 33 to 38, wherein the determination step involves selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probes.

40. An assay according to any one of claims 33 to 39, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.
- 5 41. An assay according to any one of claims 33 to 40, wherein the disease or condition to be assayed is selected from cancers.
42. An assay according to claim 41, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and
10 liver cancer.
43. An assay according to claim 42, wherein the disease or condition to be assayed is prostate cancer.
- 15 44. An assay according to claim 42, wherein the disease or condition to be assayed is liver cancer.
45. A primer or probe comprising a nucleotide sequence selected from the group consisting of:
- 20 CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)
CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)
YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)
YGGYGYGTTAGTTYGTTGYGTATATTTTC (SEQ ID NO: 4)
GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)
25 TTTTATAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)
GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)
AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 8)
AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 9)
TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 10)
30 GAAACGCTCCGAACCCCTAAAAACCGCTAACG (SEQ ID NO: 11)

- CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 12)
ACCCCRACRACCRCTACACCCCRACGTCG (SEQ ID NO: 13)
CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 14)
AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 15)
5 AACTCCCRCCGACCCCAACCCCGACGACCG, (SEQ ID NO: 16),

wherein Y is a mixture of C and T, and R is a mixture of A and G.

46. A probe comprising a nucleotide sequence selected from the group consisting of:

10 Conversion oligonucleotide:

- AAACCTAAAAAATAAACAAACAA (SEQ ID NO: 17)
GGGCCTAGGGAGTAAACAGACAG (SEQ ID NO: 18)
CCTTTCCCTCTTTCCCARRTCCCCA (SEQ ID NO: 19)
TTTGGTATTTTTTTTCGGGTTTTAG (SEQ ID NO: 20)
15 CTTGGCATCCTCCCCCGGGCTCCAG (SEQ ID NO: 21)
GGYAGGGAAGGGAGGYAGGGGYTGGG (SEQ ID NO: 22).

Sequence Listings:

Applicant: Commonwealth Scientific and Industrial Research
Organisation

Title: Diagnostic assay

Prior Application Number: PP3129

Prior Application Filing Date: 1998-04-23

Number of SEQ ID NOs: 59

Software: PatentIn Ver. 2.1

SEQ ID NO: 1

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 1

cgcgagggtt tcgttgaggt ttcgtcgtc

29

SEQ ID NO: 2

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 2

cggttattagt gactacgcgc ggttc

25

SEQ ID NO: 3

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 3

yggtttttagg gaattttttt tcgc

24

SEQ ID NO: 4

Length: 28

Type: DNA

Organism: Homo sapiens

Sequence: 4

ygggygygtta gtttggttgyg tatatttc

28

SEQ ID NO: 5

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 5

gggaattttt tttcgcgatg ttttyggcgc

29

SEQ ID NO: 6

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 6

tttttagggg gtttyggagcg tttc

24

SEQ ID NO: 7

Length: 19

Type: DNA

Organism: Homo sapiens

Sequence: 7

ggtaggttgy gtttatcgc

19

SEQ ID NO: 8

47

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 8

aaaaattcra atctctccga ataaacg

27

SEQ ID NO: 9

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 9

aaaaaccraa ataaaaacca cacgacg

27

SEQ ID NO: 10

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 10

tcccatccct ccccgaaacg ctccg

25

SEQ ID NO: 11

Length: 33

Type: DNA

Organism: Homo sapiens

Sequence: 11

gaaacgctcc gaaccccta aaaaccgcta acg

33

SEQ ID NO: 12

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 12

crcctaaaa tccccraaat crcgcg

27

SEQ ID NO: 13

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 13

acccracra ccrctacacc ccraacgtcg

30

SEQ ID NO: 14

Length: 31

Type: DNA

Organism: Homo sapiens

Sequence: 14

ctcttctaaa aaatcccr cr aactcccgcc g

31

SEQ ID NO: 15

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 15

aaaacrcctt aaaatccccg aaatcgccg

29

SEQ ID NO: 16

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 16

aactcccrcc gacccaacc ccgacgaccg

30

SEQ ID NO: 17

Length: 23

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds bisulfite-converted human GST-Pi gene

Sequence: 17

aaacctaataa aataaacaaa caa

23

SEQ ID NO: 18

Length: 23

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds non-converted human GST-Pi gene

Sequence: 18

gggcctaggg agtaaacaga cag

23

SEQ ID NO: 19

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds human GST-Pi gene

Sequence: 19

cctttccctc tttcccarrrt cccca

25

SEQ ID NO: 20

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds bisulfite-converted human GST-Pi gene

Sequence: 20

tttggtatrrr tttttcgggt tttag

25

SEQ ID NO: 21

Length: 25

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial

Sequence:Oligonucleotide

which binds non-converted human GST-Pi gene

Sequence: 21

cttggcatcc tcccccgggc tccag

25

SEQ ID NO: 22

Length: 26

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial
Sequence: Oligonucleotide

which binds human GST-Pi gene

Sequence: 22

gggaggggaag ggaggyaggg gytggg

26

SEQ ID NO: 23

Length: 31

Type: DNA

Organism: Homo sapiens

Sequence: 23

ttatgtaata aatttgata tttgtatat g

31

SEQ ID NO: 24

Length: 25

Type: DNA

Organism: Homo sapiens

Sequence: 24

tgtagattat ttaaggtag gagtt

25

SEQ ID NO: 25

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 25

aaacctaataa aataaataa caacaaa

27

SEQ ID NO: 26

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 26

aaaaaacctt tccctctttc ccaaatecc

29

SEQ ID NO: 27

Length: 27

Type: DNA

Organism: Homo sapiens

Sequence: 27

tttggtggtt gtttatTTTT taggttt

27

SEQ ID NO: 28

Length: 26

Type: DNA

Organism: Homo sapiens

Sequence: 28

gggatttggg aaagaggga aggttt

26

SEQ ID NO: 29

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 29

actaaaaact ctaaacccca tccc

24

SEQ ID NO: 30

Length: 24

Type: DNA

Organism: Homo sapiens

Sequence: 30

aacctaatac taccttaacc ccat

24

SEQ ID NO: 31
Length: 33
Type: DNA
Organism: Homo sapiens

Sequence: 31
aatcctcttc ctactatcta ttactccct aaa 33

SEQ ID NO: 32
Length: 29
Type: DNA
Organism: Homo sapiens

Sequence: 32
aaaacctaaa aaaaaaaaaa aaacttccc 29

SEQ ID NO: 33
Length: 29
Type: DNA
Organism: Homo sapiens

Sequence: 33
ttggttttat gttgggagtt ttgagtttt 29

SEQ ID NO: 34
Length: 29
Type: DNA
Organism: Homo sapiens

Sequence: 34
ttttgtgggg agttggggtt tgatgttgt 29

SEQ ID NO: 35
Length: 29
Type: DNA
Organism: Homo sapiens

Sequence: 35
ggtttagagt ttttagtatg gggttaatt 29

SEQ ID NO: 36
Length: 20
Type: DNA
Organism: Homo sapiens

Sequence: 36
tagtattagg ttagggtttt 20

SEQ ID NO: 37
Length: 29
Type: DNA
Organism: Homo sapiens

Sequence: 37
aactctaacc ctaatctacc aacaacata 29

SEQ ID NO: 38
Length: 29
Type: DNA
Organism: Homo sapiens

Sequence: 38
caaaaaactt taaataaacc ctcctacca 29

SEQ ID NO: 39
Length: 32
Type: DNA
Organism: Homo sapiens

Sequence: 39
gttttggtgt taggttggtt ttaggtgtt ag 32

SEQ ID NO: 40
Length: 30
Type: DNA
Organism: Homo sapiens

Sequence: 40
gttttgagta tttgttgtgt ggtagttttt 30

SEQ ID NO: 41
Length: 30
Type: DNA
Organism: Homo sapiens

Sequence: 41
ttaatatataaa taaaaaaaaat atattttacaa 30

SEQ ID NO: 42
Length: 34
Type: DNA
Organism: Homo sapiens

Sequence: 42
caaccccccaa taccacaaccc taatacaaat actc 34

SEQ ID NO: 43
Length: 26
Type: DNA
Organism: Homo sapiens

Sequence: 43
ggtttttagtt tttggttggtt tggatg 26

SEQ ID NO: 44
Length: 26
Type: DNA

Organism: Homo sapiens

Sequence: 44

tttttttgtt tttagtatat gtgggg

26

SEQ ID NO: 45

Length: 30

Type: DNA

Organism: Homo sapiens

Sequence: 45

atactaaaaa aactattttc taatcctcta

30

SEQ ID NO: 46

Length: 29

Type: DNA

Organism: Homo sapiens

Sequence: 46

ccaaactaaa aactccaaaa aaccactaa

29

SEQ ID NO: 47

Length: 38

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human
GST-Pi oligonucleotide

Sequence: 47

tgtaaaacga cggccagtgg gatttgggaa agagggaa

38

SEQ ID NO: 48

Length: 38

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human
GST-Pi oligonucleotide

Sequence: 48

tgtaaaacga cggccagttg ttgggagttt tgagtttt 38

SEQ ID NO: 49

Length: 31

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human
GST-Pi oligonucleotide

Sequence: 49

tgtaaaacga cggccagtta gtattaggtt a 31

SEQ ID NO: 50

Length: 37

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human
GST-Pi oligonucleotide

Sequence: 50

tgtaaaacga cggccagtgt tttgagtatt tgttgtg 37

SEQ ID NO: 51

Length: 35

Type: DNA

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: M13-human
GST-Pi oligonucleotide

Sequence: 51

tgtaaaacga cggccagtgt ttttagtata tgtgg

35

SEQ ID NO: 52

Length: 499

Type: DNA

Organism: Homo sapiens

Sequence: 52

tgcagatcac ctaagggtcag gagttcgaga ccagcccggc caacatggtg aaaccccgtc 60
tctactaaaa atacaaaaat cagccagatg tggcacgcac ctataattcc acctactcgg 120
gaggctgaag cagaattgct tgaacccgag aggcggaggt tgcagtgagc cgccgagatc 180
gcgccactgc actccagcct gggccacagc gtgagactac gtcataaaat aaaataaaat 240
aacacaaaat aaaataaaat aaaataaaat aaaataaaat aataaaataa aataaaataa 300
aataaaataa aataaaataa agcaatttcc tttcctctaa gcggcctcca cccctctccc 360
ctgccctgtg aagcgggtgt gcaagctccg ggatcgcagc ggtcttaggg aatttcccc 420
cgcgatgtcc cggcgcgcca gttcgctgcg cacacttcgc tgcggtcctc ttcctgctgt 480
ctgtttactc cctaggccc 499

SEQ ID NO: 53

Length: 316

Type: DNA

Organism: Homo sapiens

Sequence: 53

gggacctggg aaagagggaa aggettcccc ggccagctgc gcggcgactc cggggactcc 60
agggcgcccc tctgcggccg acgcccgggg tgcagcggcc gccggggctg gggccggcgg 120
gagtccgcgg gaccctccag aagagcggcc ggcgccgtga ctcagcactg gggcggagcg 180
gggcgggacc acccttataa ggctcggagg ccgcgaggcc ttcgctggag tttcgccgcc 240
gcagtcttcg ccaccagtga gtacgcgcgg cccgcgtccc cggggatggg gctcagagct 300

cccagcatgg ggccaa

316

SEQ ID NO: 54

Length: 603

Type: DNA

Organism: Homo sapiens

Sequence: 54

```
cagcatcagg ccggtgctcc cggcagggct cctcgccac ctcgagaccc gggacggggg 60
cctaggggac ccaggacgtc ccagtgccg ttagcggctt tcagggggcc cggagcgcct 120
cggggagggg tgggaccccg ggggcgggga gggggggcag gctgcgctca ccgcgccttg 180
gcctcctccc ccgggctcca gcaaactttt ctttgctcgc tgcagtgccg ccctacaccg 240
tggtctatct ccagttcga ggtaggagca tgtgtctggc aggggaaggga ggcaggggct 300
ggggtgcag ccacagccc ctcgcccacc cggagagatc cgaaccccct tatccctccg 360
tcgtgtggct ttaccccgg gcctccttcc tgttccccgc ctctcccgcc atgcctgttc 420
ccgccccag tgttgtgtga aatcttcgga ggaacctgtt tacctgttcc ctccctgcac 480
tcctgacccc tccccgggtt gctgcgaggc ggagtcggcc cgggtccccac atctcgtact 540
tctccctccc cgcaggccgc tgcgcggccc tgcgcatgct gctggcagat cagggccaga 600
gct 603
```

SEQ ID NO: 55

Length: 266

Type: DNA

Organism: Homo sapiens

Sequence: 55

```
gctctgagca cctgctgtgt ggcagtctct catccttcca cgcacatcct cttcccctcc 60
tcccaggctg gggctcacag acagccccct ggttgggcca tcccagtgga ctgtgtgttg 120
atcaggcgcc cagtcacgcg gcctgctccc ctccacccaa ccccagggct ctatgggaag 180
gaccagcagg aggcagccct ggtggacatg gtgaatgacg gcgtggagga cctccgctgc 240
aaatacatct cctcatcta caccaa 266
```

SEQ ID NO: 56

Length: 287

Type: DNA

Organism: Homo sapiens

Sequence: 56

```
tccccctgct ctcagcatat gtggggcgcc tcagtgcccg gcccaagctc aaggccttcc 60
tggcctcccc tgagtacgtg aacctcccca tcaatggcaa cgggaaacag tgagggttgg 120
ggggactctg agcgggaggc agagtttgcc ttcctttctc caggaccaat aaaatttcta 180
agagagctac tatgagcact gtgtttcctg ggacgggggt taggggttct cagcctcgag 240
gtcggtgga ggcagagca gaggactaga aaacagctcc tccagca 287
```

SEQ ID NO: 57

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 57

```
ataaaataaaa ataaaataaaa ataaagcaat ttcctttcct ctaagcggcc tccacccctc 60
tcccctgccc tgtgaagcgg gtgtgcaagc tccgggatcg cagcggctct aggggaatttc 120
cccccgcat gtcccggcgc gccagttcgc tgcgcacact tcgctgcggt cctcttcctg 180
ctgtctgttt actccctagg ccccgctggg gacctgggaa agagggaaag gcttccccgg 240
ccagctgcgc ggcgactccg gggactccag ggcgcccctc tgcggccgac gcccggggtg 300
cagcggccgc cggggctggg gccggcggga gtccgcggga ccctccagaa gagcggccgg 360
cgccgtgact cagcactggg gcggagcggg gcgggaccac ccttataagg ctcgagggcc 420
gcgaggcctt cgctggagtt tcgcgcgcgc agtcttcgcc accagtgagt acgcgcggcc 480
cgcgctccccg gggatggggc tcagagctcc cagcatgggg ccaa 524
```

SEQ ID NO: 58

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 58

```
ataaaataaaa ataaaataaaa ataaagtaat tttttttttt ttaagtgggt tttatttttt 60
ttttttgttt tgtgaagtgg gtgtgtaagt tttgggattg tagtggtttt aggggaatttt 120
tttttgtgat gttttggtgt gttagtttgt tgtgtatatt ttgttggtgt tttttttttg 180
ttgtttgttt attttttagg ttttgttggg gatttgggaa agagggaaag gtttttttgg 240
ttagtttgtt ggtgattttg gggatttttag ggtgtttttt tgtggttgat gtttggggtg 300
tagtggttgt tgggggttggg gttggtggga gtttgtggga ttttttagaa gagtgggttg 360
```


tgttgtgatt tagtattggg gtggagtggg gtgggattat ttttataagg tttggaggtt 420
gtgaggtttt tgttgaggtt ttgttggtgt agtttttggt attagtgagt atgtgtgggt 480
tgtgtttttg gggatggggg ttagagtttt tagtatgggg ttaa 524

SEQ ID NO: 59

Length: 524

Type: DNA

Organism: Homo sapiens

Sequence: 59

ataaaataaa ataaaataaa ataaagtaat tttttttttt ttaagcgggt tttatttttt 60
ttttttgttt tgtgaagcgg gtgtgtaagt ttcgggatcg tagcggtttt aggggaatttt 120
ttttcgcgar gtttcggcgc gttagttcgt tgcgtatatt tcggtgcggt tttttttttg 180
ttgtttgttt attttttagg tttcgttggg gatttgggaa agagggaaaag gtttttttcgg 240
ttagttgcgc ggcgatttcg gggatttttag ggcgtttttt tgcggtcgac gttcgggggtg 300
tagcggtcgt cgggggttggg gtcggcgagg gttcgcggga ttttttagaa gagcggtcgg 360
cgtcgtgatt tagtattggg gcggagcggg gcgggattat ttttataagg ttcggaggtc 420
gcgaggtttt cgttggagtt tcgtcgtcgt agttttcgtt attagtgagt acgcgcgggt 480
cgcgttttcg gggatggggg ttagagtttt tagtatgggg ttaa 524